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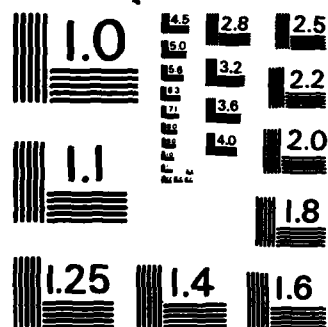
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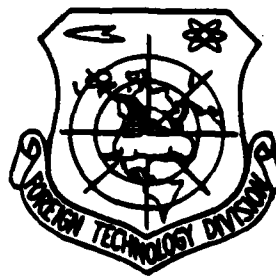
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THE ABSORPTION OF BENZENE THROUGH HUMAN SKIN

by

J. Hanke, T. Dutkiewicz, J. Piotrowski



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THE ABSORPTION OF BENZENE THROUGH HUMAN SKIN

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It is common knowledge that many organic substances are capable of being absorbed through the uninjured skin. The role of skin in absorption is overemphasized or, on the contrary, neglected, depending on the amount of clinical information gathered on poisonings caused by absorption of a given toxic substance through the skin. As far as benzene is concerned, the generally accepted view is that it is capable of penetrating the skin, although the degree is insufficient to cause poisoning. In this connection, the accepted opinion in practice is that the only significant way of benzene penetration in industrial circumstances is through the lungs.

As a result of the proliferation of benzene solutions in several production technologies and prolonged exposure poisonings, the question about skin as a way of absorption has re-emerged with respect to the direction of preventive measures and the necessity to clarify the observed clinical signs of absorption. (R. Vlasak, 1959).

Available information about the absorption of benzene through the skin is primarily based on two experimental works with contradicting results.

Lazarev, Drussilkovskaja and Lavrov made experiments on rabbits and mice (1931). Skin contact with fluid benzene was established through dipping the ears and feet of animals in liquid benzene during several hours. The amount of absorbed benzene was considered to be the amount of benzene exhaled by the animals determined by burning it into carbon dioxide and measured with the help of conductometric analysis of the absorbent material.

The speed of liquid benzene absorption was determined to be $0.05 \text{ mg/cm}^2/\text{min}$ (i.e. $3 \text{ mg/cm}^2/\text{hour}$). Such a rate of absorption had to be taken into consideration in practice, especially because the authors believed the results to be too low in relation to reality because no other way of benzene emission from the animals' bodies was noted.

The same authors analyzed also the absorption of benzene vapors through animal skin, securing that no benzene vapor was inhaled. The number of phenol particles in the urine of the experimental animals was accepted to be the orientational index of absorption. The results of the experiments were interpreted to be of positive quality.

There are two basic arguments against the mechanical adoption of these experimental results for industrial workers contacting benzene:

a) The implied method of exposing the animals to benzene caused far reaching changes in the physiological state of the skin, totally mummifying the exposed parts. The results received in such conditions cannot be interpreted as absorption through unharmed skin.

b) We have to assume that there are serious differences between the absorption characteristics of human and animal skin, due to the different anatomical structure and function of the skin.

As a result of the above opinions, the question was raised again by N. Caesaro (1946) who made absorption experiments on humans with liquid and vaporized benzene. In the case of liquid benzene he covered shoulders with cotton impregnated with benzene.

a) The amount of exhaled benzene vapor and, b) the decrease*

* As a result of the chemical reaction of phenol with an ester of sulfuric acid the amount of sulfuric ester rises at the expense of non-organic sulfates. It causes a drop in the whole sulfate ratio which is approx. 75-95% under physiological conditions.

of the so-called sulphate ratio in the urine were accepted as an index for the absorbed amount of benzene. Both indices gave negative results; therefore, the author came to the conclusion that the absorption of benzene through the skin did not figure among industrial risks. One should have reservations about the index of benzene absorption used in that experiment because, as it is known, the index is not sensitive enough. Obvious reduction in the sulphate index can be observed only when the benzene vapor absorption of the lungs is close to the industrial norm, i.e., 0.1 mg/l².* Negative results in the sulphate tests cannot be regarded as sufficiently negative evidence in the discussion about benzene absorption.

Teisinger and Fiserova-Bergerova elaborated (1955) an interpretation concerning benzene absorption based on phenol contents in urine. This ratio is very sensitive and makes the measurement excretion in urine (as a result of absorption of trace benzene amounts) feasible.

The present work is aimed at the quantitative description of the speed of benzene absorption through human skin. We tried to make a reliable evaluation about the role skin plays in absorbing benzene in industrial circumstances.

We relief in our work on the following absorption criteria:

- 1) Phenol separation in given urine fractions during a period of time not less than a day. The interpretation of the results was carried out according to the data of Teisinger and Fiserova-Bergerova.

- 2) Chemical determination of the amount of the absorbed liquid benzene through the following method, elaborated especially for this purpose.

*This norm is actually considered to be too high. GOST of 1959 allows 0.02 mg/l of precipitation.

The experiments covered

- a) the absorption of liquid benzene given through an isolated napkin or container (depending on the applied method of evaluation),
- b) absorption of benzene vapors, whereas phenol excretion in urine was accepted as an absorption index.

Methodology of the experiments

1. Direct measuring of benzene absorption through the skin

A method was elaborated which can be used not only in experiments concerning the speed of benzene absorption, but also with other vaporizing solvents. The principle of the method does not differ from that previously used in our factory to experiment with less vaporizing compounds: anilyn (J. Piotrowski, 1957) and nitrobenzene (J. Salmowa and J. Piotrowski, 1960). A precisely measured amount of chemical substance is placed on the skin. The chemical substance makes contact with a given skin surface, during a known period of time. The amount of absorbed chemical substance can be determined from the difference left after having determined the non-absorbed amount. The same method can be applied for calculating the speed of absorption. The successful prevention of loss caused by vaporization during the experiment has been a precondition for using the above method.

In the case of benzene, insulation protection against vaporization turned out to be unsatisfactory due to its high volatility. The novelty of the recently elaborated method lies in the utilization of volatility of the studied substance as a basic in determining absorption.

This method is used in the following way:

A small amount (0.02 ml) of benzene is placed on a watch-glass using a small hypodermic needle. The watch-glass is consequently fixed on the skin of the forearm with a rubber band.

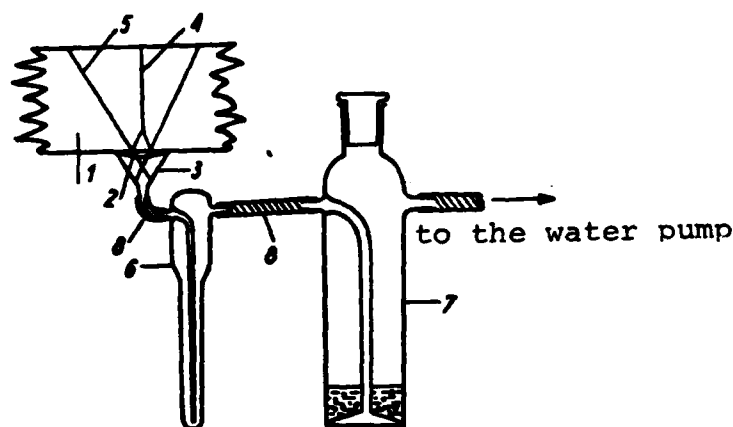


Figure 1. Scheme of the system designed to measure directly the absorption of liquid benzene through the human skin.

1--lower arm; 2--watch glass; 3--glass funnel; 4--rubber band pressing the watch glass against the skin; 5--rubber band to hold the funnel on the forearm; 6--buffer tub (empty); 7--rinsing tub containing the nitrating mixture; 8--connecting rubber pipes

The watch glass is placed under a funnel with a somewhat larger diameter. The funnel is connected by short rubber pipes to a water suction pump through a rinsing tub containing porous glass. The rinsing tub contains 20 ml of nitrating mixture (10% of ammonia nitrate in sulphur acid concentrate) acting as an absorbing agent. The system has a constant air flow passing over the glass at a speed of 0.5-1 l/min (Figure 1). When the experiment is over, the rubber band pressing the watch glass to the forearm is cut through causing a swift evaporation of benzene. An air stream is conducted for 15 minutes after the rubber band is cut. It is enough to have the extra amount of benzene evaporated and to have the system rinsed with air. The phases of the process are illustrated in Figure 2.

A similar experiment is carried out in the glass unit itself (without the presence of the person participating in the experiment) in order to determine the amount corresponding to the amount of the applied benzene. The benzene absorbed in the rinsing glass in the form of m-dinitrobenzene is absorptiometrically denoted by acetone and alkali. For this purpose, the contents of the rinsing glass

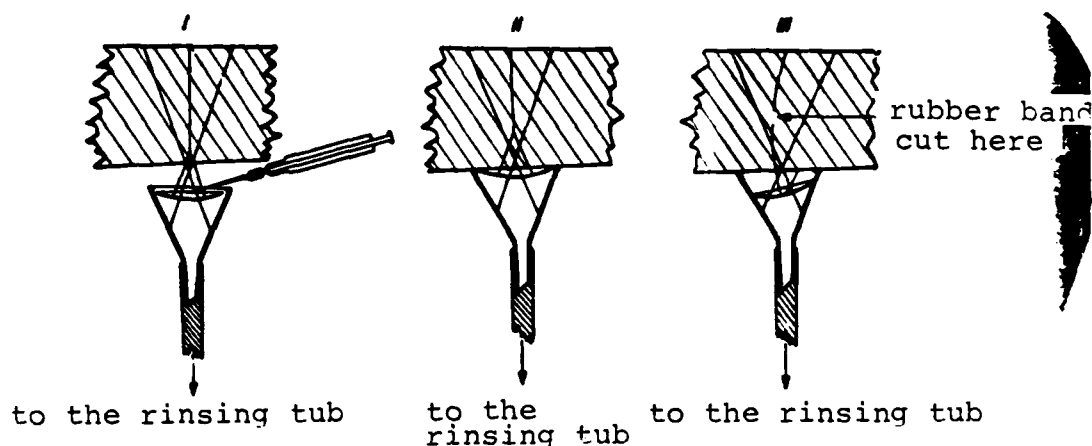


Figure 2. The operational sequence of experiments on benzene absorption through the skin.

I--benzene application with a hypodermic needle; II--absorption from the skin; III--evaporation of excess benzene

are set aside until the next day to have the nitration finished. Later it is mixed in 1 liter of distilled water. After careful mixing 20 ml of water solution is taken from the testtube for analysis.

The results of the test made with the same glass unit directly correspond to the applied amount. The amount of benzene remaining on the watch glass and the skin after the experiment can be calculated from the following formula:

$$m = \frac{A \cdot m_o}{A_o}$$

where m_o = the amount of benzene applied on the skin (in mg)

A_o = absorption in the experiment carried out in the glass unit

A = absorption in the experiment carried out on the skin

The amount of absorbed benzene ($m_o - m$) is divided by the surface of the watch glass (cm^2) and the time of skin contact (hour). The obtained result is expressed in the form of absorption speed: ($\text{mg}/\text{cm}^2/\text{hour}$). The correctness of the method was checked the following way: a measured amount of benzene was put on the watch glass and skin contact was established. After that, the contact

was immediately cut off and the benzene was vaporized. That way the exposition time of approximately $t=0$ was obtained. The results of this process did not differ from the indications in the glass unit itself, so they were included in the calibration of the method. The precision of determining the outcoming amount of benzene is $\pm 5\%$. This deviation is mainly connected with the final absorptiometric method of measurement. It is impossible to receive better results while using this method.

The following conditions have been taken into consideration in order to obtain unambiguous results (given the unsatisfactory precision of each measurement).

- 1) The experiment was conducted in such a way that the absorbed amount totaled approximately 20% of the applied volume. Under these circumstances, the obtained difference (before and after) was sufficiently big, and the losses of the chemical substance on the skin had not yet basically changed the circumstances of the absorption itself. In this connection, the amount applied on the skin had to be decreased to a minimum which made us substitute the previously used sheets for a tightly connected watch glass.

- 2) The reading of the outcoming benzene from the applied amount was repeated several times in order to decrease the mistakes connected with it. As a basis for calculations, an average with standard deviation of $\pm 1.4\%$ was accepted.

- 3) The results of the experiments on benzene absorption through the skin were not interpreted individually. An average with a sufficiently small standard error (in this experiment $\pm 2\%$) was accepted as a basis.

Since the above described circumstances were observed, the following results of the experiments can, in our opinion, be considered as reliable.

2. The measurement of benzene in the air

Both in the above described method and in the measuring of benzene vapor particles in the air of the test chamber, the absorptiometrical method was used in the Piotrowski version (1954) with acetone and KOH. The measurement was realized on a Coleman Junior spectrophotometer at 570 mμ wavelength. It was concluded that the precision of the measurement with this method was ±4%.

3. Measurement of phenol in urine and physiological values

Colorimetric method with 2.6 dibromoquinonechloroimide was used to measure phenol in urine in the version described in the handbook by Teisinger, et al. (1956). In this method, phenol is measured after being distilled from the urine from an acidic environment as whole (free or esther) phenol.

During the first experiments, the given coefficient value as indicated in the recipe turned out to be insufficient to correspond to Beer's rule in a rather large range of phenol precipitation. This ratio for 50 μg phenol is given in Figure 3. On the basis of the curve in Figure 3, the value of the coefficient was determined for 0.25 ml. The whole measurement process took place in the following way:

5 ml of the examined urine mixed together with 0.5 ml concentrated sulfuric acid is distilled in a glass apparatus (through pyrite?) with water vapor of 10 ml/minute in order to receive 50 ml destilate. An optimum amount of destilate (2.5-10 ml) was put into a 25 ml testtube. 2.5 ml buffer having 10.15 pH is added. The buffer consists of 10.5 g $\text{Na}_2\text{CO}_3 \cdot 10 \text{ H}_2\text{O}$ plus 2.8 g dehydrated borax dissolved in 0.5 l water, and 0.25 ml of 2.6 dibromoquinonechloroimide dissolved in alcohol (15 mg/10 ml) and water was added to make the solution 25 ml. An hour later measurements were made on the Coleman Junior equipment (cuvette size 25x105 mm; wavelength 610 mμ).

The calibration of the method* was made in the range of up to 30 gamma phenol in the sample; accuracy of the measurement $\pm 6\%$.

Table 1 shows the results of phenol precipitation from the urine of several people. The results were obtained while using the above method.

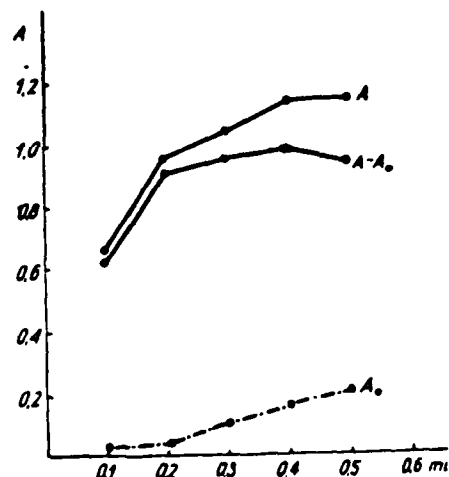


Figure 3. Absorption of solutions containing $50 \mu\text{g}$ of phenol at variable agent (2.6 dibromoquinonechloroimide) amounts. Measurements compared to water:
 A_o -- absorption of phenol-free solutions;
 A -- test absorption;
 $A-A_o$ -- increase of absorption compared to final absorption of the agents

TABLE 1. Physiological values of phenol separation in different non-exposed people

	no. of measurements	results of the measurements		
		from	to	average
phenol precipitation, mg/l	52	3.0	16.0	7.2 ± 0.44
speed of phenol separation, mg/l	32	0.08	0.57	0.24 ± 0.017

* In the example given in the quoted textbook by Teisinger, et al. concerning the preparation of phenol test solutions (Page 74, Section 1), there is a miscalculation: A solution prepared according to this recipe would have 25 gamma/ml and not 10 of precipitation.

PROCESS AND RESULTS OF THE EXPERIMENTS

1. Absorption of liquid benzene

A. Experiments on absorption speed based on phenol precipitation in the urine

Although five experiments were carried out the following way: a cotton sheet impregnated with benzene (approx. 0.06 g/cm^2) was placed on the bare forearm of the exposed person*. The cotton sheet was isolated with the help of cellophane stuck to the forearm with tape. The contacted surface was $35\text{--}45 \text{ cm}^2$, the time of contact was 1.25-2 hours. In order to eliminate the inhalation of trace amounts of benzene (from the impregnated sheet), the forearm was placed under a suction device working at high speed. Benzene contents in the air as well as fecal matter were double-checked. The results of these measurements were negative. Urine samples were taken over 24 hours; during the daytime, every 1-3 hours, and less frequently during the night.

Phenol precipitation in the urine. Phenol separation in the urine was low in these experiments. Daily amounts of separated phenol were between 8.0 and 14.7 mg. These amounts are slightly higher than the average values and are completely within the limits of physiological separation as given by Teisinger, et al. (average 8 maximum 20 mg/day).

The physiological values obtained by us were expressed either as precipitation (mg/l) or separation speed (mg/hour). Below, we give the most extreme values obtained during the absorption experiments compared to the extreme physiological values obtained by us (Table II).

* The data were obtained from self-experimentation or experiments on volunteers of the Institute of Labor Medicine.

TABLE II. Highest values of phenol separation in the urine of non-exposed people and people exposed to skin contact with liquid benzene.

	highest values	
	precipitation mg/l	separation speed mg/h
physiological separation	16.0	0.57
separation in exposed persons	15.8	0.79
	17.0	1.7
	28.3	0.96
	22.1	1.9
	16.9	0.75

As we can see from Table II, the maximum phenol separation speed in the exposed persons was considerably higher than the relative physiological values obtained by us. The increase in phenol precipitation in the urine after the exposure is demonstrated most explicitly while comparing the separation curves (see Figure 4). This figure shows three curves representing changes in phenol separation in the urine, the speed of urine separation (A) and their product: changes in phenol precipitation speed (B). This latter seems to be the most characteristic curve.

Although the fact of higher phenol separation in the urine after skin exposure to liquid benzene seems to be unquestionable, the correct measurement of excess phenol caused by benzene absorption is difficult. It is connected with the large variance of physiological values (as an example, see Figure 5). The acceptance of pre-exposure data of separation speed seems to be unjustified since the number of such measurements is low. The average pre- and post-exposure values are certainly somewhat high, since we have data obtained after only one day elapsed, when the separation had not yet reached physiological values.

The acceptance of an average value determined from a large number of experiments on different people does not seem right since

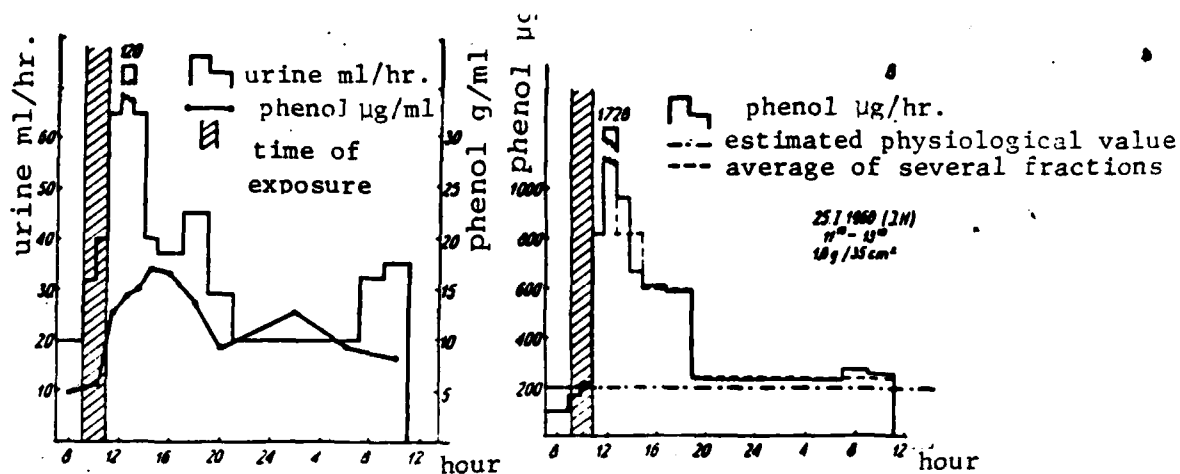


Figure 4. The process of phenol separation in the urine of a person whose skin absorbed liquid benzene.

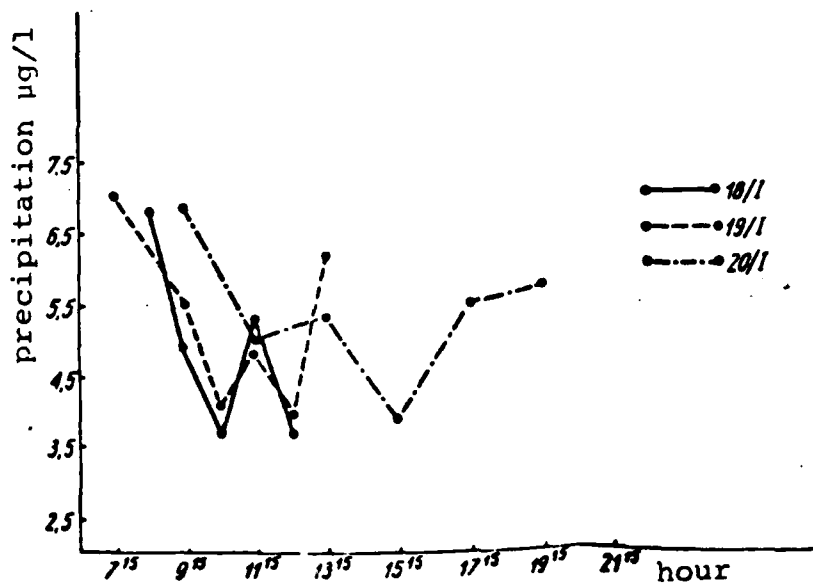


Figure 5. The fluctuation of phenol separation in consecutive urine samples of the same person.

that average (0.24 mg/hour) exceeds considerably the average of pre-exposure values obtained from the participants of the experiment (0.16 mg/hour).

It goes without saying that one could positively argue in favor of each method. It seemed to us that the results obtained would contain the least amount of error if we calculate physiological separation as an average of all four calculation methods. The results obtained this way are shown in Table III.

TABLE III. Excess phenol separation in persons exposed to skin contact with phenol

quantity of daily phenol separation in mg			
no. of persons	physiological	post experiment	excess
1	4.1	8.0	3.9
2	4.8	10.8	6.0
3	5.4	10.3	4.9
4	6.1	14.7	8.6
5	4.0	9.2	5.2

As a basis for calculating the amount of absorbed benzene, we accepted Teisinger, et al. (1956); that approximately an average of 30% of the benzene absorbed is separated in the urine in the form of phenol. In each case, the speed of liquid benzene absorption through the skin was calculated based on the above premise (see Table IV). The average speed of benzene absorption through the skin calculated this way is approximately $0.24 \text{ mg/cm}^2/\text{hour}$. This value should be regarded only as orientational taking into account the large number of stipulations made during the calculation.

Some basic data concerning the characteristics of the separation of phenol metabolite was additionally collected during the experiment of phenol precipitation. We were interested in the speed of separation, characterized by a "k" constant of the linear

TABLE IV. The calculation of liquid benzene absorption through the skin based on phenol separation in the urine

no. of persons	excess phenol in mg.	benzene equivalent in mg	surface contacted in cm ²	time of cont. (hour)	absorption speed in mg/cm ² /hr.
1	3.9	13	35	2.25	0.16
2	6.0	20	35	2	0.29
3	4.9	16	35	2	0.23
4	8.6	28	45	2	0.32
5	5.2	17	40	2	0.21

average absorption speed 0.24

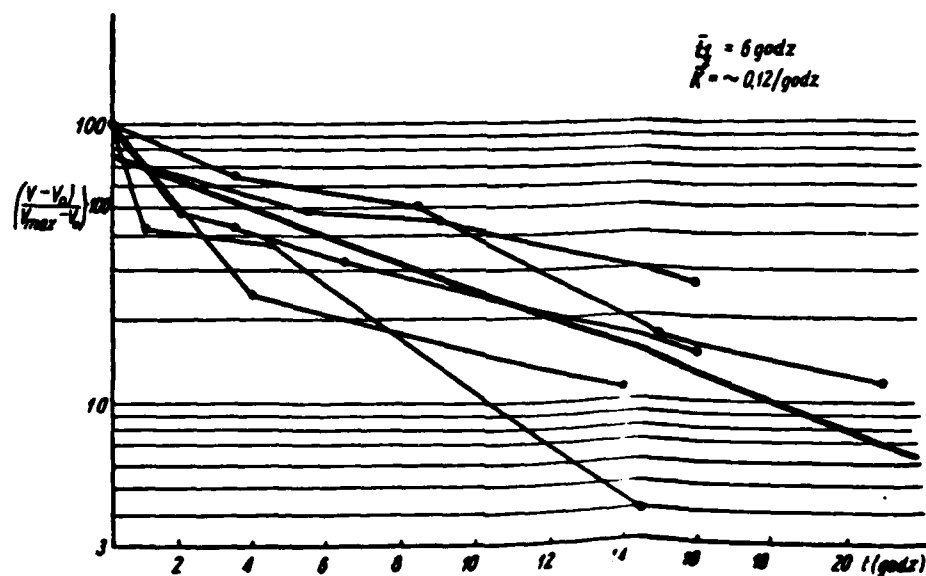


Figure 6. Excess phenol separation shown in a half-log scale time diagram. Values relating to the highest speed of separation ($V_{\max} - V_0$) are the bases. They are expressed in %.

equation. The calculations were based on a statistically determined regression model (Figure 6). The separation constant averaged around 0.12/hour, e.g., it is of the same order as the constants of metabolite separation of other aromatic compounds we studied (p-aminophenol, aniline or nitrobenzene added, p-nitrophenol with nitrobenzene given to the experimental animals). This value is only orientational since it was obtained from an insufficient amount of data and only from very small doses of benzene. One cannot exclude the possibility of a two-step phenol separation, where the drop in the speed of separation is larger during the first phase. However, the material we possess is too small and one-sided for proving this theorem beyond all doubt.

B. The measurement of the speed of liquid benzene absorption through the skin based on direct chemical measurements

The way in which this experiment was carried out was described earlier. The applied watch glass had a surface of 8 cm², 0.02 ml-17.6 mg benzene was contacting the skin for 1.25 hours. We have completed 15 measurements for calibrating and seven absorption tests on the skin of different people's forearms. Our calculations based on the data are shown in Table V.

TABLE V. Data concerning the calculation of benzene absorption (through the skin) speed based on chemical measurements.

	no. of tests	absorption values			
		from	to	average	difference
I. calibration	15	0.455	0.530	0.490 0.007	--
II. absorption	7	0.365	0.435	0.380 0.027	0.110

The average benzene loss from the watch glass caused by benzene absorption through the skin was:

$$x = \frac{0.110}{0.490} \cdot 17.6 \text{ mg} = 4.0 \text{ mg.}$$

The average absorption speed, taking into consideration the surface and contact time was:

$$v = \frac{4}{8 \times 1.25} = 0.4 \text{ mg/cm}^2/\text{hour}$$

The results obtained directly through the chemical method (0.4 mg/cm²/hour) and indirectly through phenol precipitation in the urine of exposed people (0.24 mg/cm²/hour) are suitably conformable. We think, however, that the first result (0.4) is quantitatively more reliable from the point of view of methodology since it was directly obtained. It should be mentioned that the given results concerning the speed of liquid benzene absorption through the skin represent extreme cases since the skin of exposed people was entirely soaked in benzene at the contact area, therefore, they are maximum values.

2. Absorption of benzene vapor through the skin

Tests on benzene vapor absorption through the skin were made in a special toxicology chamber meant for these types of experiments (Dutkiewicz, 1960).

Together three experiments were made. The precipitation of benzene vapor was 1.0 mg/l. Checking measurements carried out hourly showed that the fluctuation in precipitation did not exceed $\pm 5\%$. The temperature in the chamber was 25°C, and humidity was approximately 35%. The people we experimented on wore no clothes in the chamber. These people took a bath before and after entering the chamber. In order to exclude simultaneous inhalation of benzene, the person being tested in the chamber was inhaling clean air supplied through a mask from outside the chamber.

The measurement of the amount of benzene vapor absorption through the skin was based on determining phenol separation in the urine. Urine samples were taken during 24 hours before the

experiment commenced in order to determine the physiological level of phenol in the urine. During and after the exposure, urine samples were taken for a whole day.

In the first test the exposure lasted 1.5 hours. No increase of phenol separation in the urine of the exposed person was noted.

In the next two tests, the exposure time was increased up to seven hours. Phenol separation during the following 24 hours increased by 2.7 mg and 4.2 mg respectively. For an average of 3 mg phenol a day, the probable amount of benzene absorbed was about 10 mg. That is a negligible amount if we take into consideration that the amount of benzene absorbed by the lungs would have been about 1500 mg^{*}, given the same benzene density in the air (1 mg/l) and 3 m³/7 hours^{**} of lung ventilation (breathing). The absorption of benzene vapor through the skin was less than 1% of the absorption through the lungs under the given conditions that amount is sufficiently small to come to the conclusion that benzene vapor does not penetrate through the skin.

Description of the results and conclusions

The speed of liquid benzene absorption through the human skin, given full moistening of the skin of the forearm with benzene is 0.4 mg/cm²/hour. This speed is of the same order as the speed of aniline and nitrobenzene absorption determined in previous works. However, given the relatively smaller toxical effect of benzene as compared to the above two compounds, the absorption speed of liquid benzene can be considered as low. It is also essential to mention that a single moistening of the skin or cloth with benzene, due to its high volatility, does not represent a long lasting

* Having taken into consideration the retention of the lungs, which is approximately 50%.

** In a sitting position, without movement.

danger as it does in the case of aniline or nitrobenzene.

In this connection, skin protection against benzene absorption should not, with the exception of a few professions, cause difficulty. It should be mentioned, however, that in those activities where the human skin is repeatedly moistened, e.g., hand-painting using paint which contain benzene thinner and similar activities where paint stains on the skin represent a relatively permanent source of benzene, benzene absorption through the skin could play a very important role. Assuming that the surface is 600 cm^2 , the maximum amount of absorption during eight hours of working could be in this case approximately 2000 mg. This amount could not cause serious poisoning and presumably that accounts for the lack of data about serious poisonings with benzene absorbed through the skin. At the same time, this is a large amount which may cause cumulative poisoning. Let us assume that the highest level of benzene vapor concentration in the air legally permitted in Poland is 0.1 mg/l . This corresponds to the absorption of almost 300 mg per day^* . The concentration allowed by GOST (0.02 mg/l) corresponds to nearly 60 mg.

The absorption of liquid benzene through the skin must not be neglected in activities involving bare hands. It should be taken into consideration at least as an additional way of absorption.

The absorption of benzene vapors does not seem to represent any toxicological problem.

The authors thank Mr. U. Neuhorn for his technical help in carrying out the experiments.

* Assuming lung ventilation is approximately $6 \text{ m}^3/8 \text{ hours}$ and benzene retention of the lungs is 50%.

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Summary

The absorption of benzene throughout the skin in men in experimental conditions was examined. The liquid benzene was absorbed with the velocity about 0.4 mg/cm²/hour; the absorption of the benzene vapors was insignificant and it did not exceed 1 per cent of this absorbed throughout the respiratory ways in the same conditions. The study was based on the determination of phenol contents in urine and on the new direct chemical method for the designation of skin absorption of benzene, this method was described in the paper.

The authors concluded that the absorption of benzene throughout the skin must not be neglected.

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